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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/723,570	11/24/2003	John M. Pawelek	869-BAZ-US	9925

7590 08/09/2005

Albert Wai-Kit Chan
Law Offices of Albert Wai-Kit Chan, LLC
141-07 20th Avenue
World Plaza, Suite 604
Whitestone, NY 11357

EXAMINER	
DUNSTON, JENNIFER ANN	
ART UNIT	PAPER NUMBER
1636	

DATE MAILED: 08/09/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/723,570	PAWELEK ET AL.	
	Examiner	Art Unit	
	Jennifer Dunston	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) 1-13 is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) 1-13 is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on 24 November 2003 is/are: a)☐ accepted or b)☒ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date 11/24/2003.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Receipt is acknowledged of an amendment, filed 11/24/2003, in which the specification was amended to update the claim for priority and to include ATCC accession numbers.

Information Disclosure Statement

Receipt of an information disclosure statement, filed on 11/24/2003, is acknowledged. The signed and initialed PTO 1449 has been mailed with this action.

Drawings

The replacement drawings for Figure 9, panels A-D were received on 6/8/2004. These drawings are acceptable.

The drawings are objected to as failing to comply with 37 CFR 1.84(p)(5) because they include the following reference character(s) not mentioned in the description: Figure 1 includes the reference character "C'", which is not described in the specification. The figure 1 legend describes reference character "D", which is not found in Figure 1. It appears as though the description of reference character "D" applies to reference character "C'". Corrected drawing sheets in compliance with 37 CFR 1.121(d), or amendment to the specification to add the reference character(s) in the description in compliance with 37 CFR 1.121(b) are required in reply to the Office action to avoid abandonment of the application. Any amended replacement drawing sheet should include all of the figures appearing on the immediate prior version of the sheet, even if only one figure is being amended. Each drawing sheet submitted after the filing

date of an application must be labeled in the top margin as either "Replacement Sheet" or "New Sheet" pursuant to 37 CFR 1.121(d). If the changes are not accepted by the examiner, the applicant will be notified and informed of any required corrective action in the next Office action. The objection to the drawings will not be held in abeyance.

Specification

The disclosure is objected to because of the following informalities:

The status of application 09/358,052 should be updated to include the phrase "currently U.S. Patent No. 6,685,935."

The page numbers on the Table of Contents pages of the specification (i-iv) do not correspond to the numbering of the rest of the specification.

The address for the ATCC depository has changed. It would be remedial to amend the specification to use the current address of the depository: P.O. Box 1549, Manassas, VA 20108.

Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-13 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in

the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Enablement is considered in view of the Wands factors (MPEP 2164.01(A)). These include: nature of the invention, breadth of the claims, guidance of the specification, the existence of working examples, state of the art, predictability of the art and the amount of experimentation necessary. All of the Wands factors have been considered with regard to the instant claims, with the most relevant factors discussed below.

Nature of the invention: The claims are drawn to a method for reducing volume or inhibiting growth of a solid tumor cancer, comprising administering to a patient having a solid tumor, a tumor specific *Escherichia coli* genetically engineered to express a suicide gene. The dependent claims limit the method to embodiments wherein the *E. coli* is attenuated, super-infective, or attenuated and super-infective. Further, the attenuated *E. coli* may expresses an altered lipid A molecule or induce TNF-α expression in monocytes or macrophages from about 1 to about 75 percent compared to non-attenuated microorganisms. The claims encompass embodiments wherein the *E. coli* is a single colony clone, an enteroinvasive *E. coli*, or an auxotrophic mutant. The suicide gene of the *E. coli* may be selected from the group consisting of the open reading frame of pTK-Sec3, pCD-Sec1 and pSP-SAD4-5 or may encode p450 oxidoreductase, HSV thymidine kinase, *E. coli* cytosine deaminase, carboxypeptidase G2, β-glucuronidase, penicillin-V-amidase, penicillin-G-amidase, β-lactamase, β-glucosidase, nitroreductase and carboxypeptidase A. The suicide gene may be under the control of a constitutive promoter, an inducible promoter, or a tumor cell specific promoter.

The nature of the invention is complex in that the genetically engineered *E. coli* must be tumor specific such that the cancerous tumor cells can be targeted with the suicide gene without causing significant harm to normal cells.

Breadth of the claims: The claims are broad in that they encompass the use of any *E. coli* strain genetically engineered to express any suicide gene to treat any solid tumor cancer. The breadth of the claims exacerbates the complex nature of the subject matter of this invention.

Guidance of the specification and existence of working examples: The specification envisions the isolation and use of super-infective tumor-specific, attenuated strains of parasites, including bacteria, for the treatment of solid tumors (e.g. page 1, lines 10-20). The specification broadly envisions the use of genetic engineering to make a parasite specific for a tumor cell (e.g. page 13, lines 20-36). The specification envisions the use of *E. coli*, among many other parasites (e.g. page 14, 1st paragraph; page 26). The specification teaches the desirability of characteristics such as antibiotic sensitivity, biospecificity, mutant isolation and genetic manipulation, chemotaxis, replication within target cells, and anaerobic and aerobic metabolism. However, the specification does not address each of these issues with regard to *E. coli* (e.g. pages 11-13). *E. coli* is a facultative anaerobe that can be made sensitive to antibiotics, can be manipulated by genetic engineering, and has been used to study chemotaxis (e.g. pages 11-13).

Biospecificity refers to the ability of the *E. coli* to express specificity for the tumor cells. The greater the specificity, the lower the inoculum necessary for effective therapy and the lower the risk of septic shock or pan-infection (e.g. page 12, 1st paragraph). The specification teaches that bacteria such as *Salmonella typhimurium*, and *Mycobacterium avium* have a natural preference for attachment to and penetration into certain solid tumor cancer cells in tissue

culture, as opposed to non-cancerous counterpart cells (e.g. page 27, 1st paragraph). The specification does not teach *E. coli* that has a natural preference for tumor cells. Conversely, the specification teaches that wild type *E. coli* are found in relatively low number in both the tumor and liver of inoculated animals, with more bacteria found in the liver as compared to the tumor (e.g. page 96, 1st full paragraph; Table 18). Sections 6.1.1-6.1.4 of the specification teach methods of isolating a microorganism with enhanced biospecificity by selecting mutagenized or un-mutagenized microorganisms that infect tumor cells *in vitro* or *in vivo* or isolating microorganisms that are capable of chemotaxis toward tumor cell conditioned medium. The ability of these methods to make super-infective *Salmonella* is taught in sections 7-9 of the instant specification. Although the examples demonstrate that they disclosed methods are capable of increasing the tumor-specificity of a microorganism with a natural ability to infect tumor cells, it is unclear as to whether the disclosed screening protocols would be sufficient to convert *E. coli* to a tumor-specific microorganism. The specification does not provide guidance with regard to specific modifications of the *E. coli*, which would result in a tumor-specific *E. coli*.

Replication within target cells refers to the ability of the microorganism to replicate within the cancer cell, resulting in an increased therapeutic effectiveness of the vector (e.g. page 13, 1st paragraph). The specification does not teach that the *E. coli* is capable of replicating in tumor cells.

No working examples are provided that demonstrate tumor volume reduction or tumor growth inhibition as a result of *E. coli* genetically engineered to express a suicide gene.

Given the small amounts of *E. coli* that are capable of infecting mammalian cells, the higher proportion of bacteria localized to the liver relative to the tumor, and the lack of guidance with regard to the ability of the *E. coli* to replicate in the tumor cell, it would require undue experimentation to make and use *E. coli* to reduce the volume or inhibit growth of a solid tumor cancer in a patient.

Predictability and state of the art: At the time the invention was made, the use of live, genetically engineered *E. coli* to treat cancer was underdeveloped and unpredictable.

Falkow (Cell, Vol. 65, pages 1099-1102, 1991, of record) teaches that the factors involved in bacterial entry into a eukaryotic cell are complex and tightly regulated and have been identified in only a few cases (e.g. page 1099, paragraph bridging columns). Further, Falkow teaches that *E. coli* possesses a receptor that allows the bacteria to adhere to the surface of the mammalian cells; however, the bacteria are not internalized efficiently (e.g. page 1100, left column, 1st full paragraph). The characteristic complexity of cell adhesion and internalization is further complicated in the instant invention in that the bacteria should preferentially adhere to and be internalized by cancer cells. Thus, the bacteria must express proteins that recognize a cell surface protein differentially expressed between a cancerous and non-cancerous cell.

Enteroinvasive *E. coli* are capable of attaching to and invading the colonic enterocytes by endocytosis and replicating within the enterocytes (Clarke, Diagnostic Microbiology and Infectious Disease, Vol. 41, pages 93-98, 2001; e.g. page 95, paragraph bridging columns). However, the infection of the colonic enterocytes results in an inflammatory response accompanied by necrosis and ulceration of the large bowel leading to release of blood and mucus in stools (Clarke, e.g. page 95, paragraph bridging columns). The biospecificity and ability of

the strain to replicate in a mammalian cell are directed toward normal colonic cells. The specification and art of record do not provide evidence that enteroinvasive *E. coli* are specific for tumor cells.

Karapetyan (EP 0564121, of record) teach three strains of *E. coli*, ATCC 55373, 55374 and 55375, which are oncolytic and hemolytic *in vitro* (e.g. page 3, line 5 to page 4, line 5; Table I). There is no description of the ability of the *E. coli* to distinguish between a cancerous target cell and a non-cancerous counterpart cell so that the vector preferentially attaches to, infects and/or remains viable in the cancer cell.

Regarding the ability of the *E. coli* to effectively reduce the volume or inhibit the growth of any type of solid tumor cancer, Jain (Exp. Opin. Biol. Ther. Vol. 1, No. 2, pages 291-300, 2001) teaches that it is unlikely that there would be an ideal anticancer agent of bacterial origin applicable to all types of cancers due to the great variations in the biology and location of various tumors (e.g. page 298, left column, 1st full paragraph).

The prior art does not appear to offset the deficiencies of the instant specification with regard to tumor specific *E. coli* that are capable of specifically attaching to and invading tumor cells without causing a significant toxic reaction in the subject at levels high enough to efficiently treat the tumor.

Amount of experimentation necessary: The quantity of the experimentation necessary to carry out the claimed invention is high as the skilled artisan could not rely on the prior art or the present specification to teach how to make and use any *E. coli* genetically engineered to express a suicide gene and able to specifically target any type of solid tumor cancer to reduce the tumor volume or inhibit tumor growth. In order to carry out the claimed invention, one would first

have to make an *E. coli* that is specific for tumor cells. Because *E. coli* do not have a natural biospecificity for tumor cells, the *E. coli* would need to be selected for biospecificity using the assays disclosed in the instant specification. However, due to the lack of any natural biospecificity for tumor cells, it is likely that one would have to mutagenize the *E. coli* prior to any screening assay. The mutagenesis may require the addition of genetic material to the *E. coli* to provide some tumor attachment and invasion activity. Because the genetic determinants of tumor specificity do not appear to be disclosed in the instant specification or art of record, one would first have to identify these genetic determinants in a tumor specific bacterium such as the *Salmonella* disclosed in the instant specification. This is expected to require a large amount of trail and error experimentation due to the underdeveloped knowledge in the area and the complex nature of mechanisms of bacterial attachment and invasion. Upon the isolation of tumor specific *E. coli* one would have to conduct experiments to verify that the invasive *E. coli* can be administered to the host without causing a severe systemic reaction. If the toxicity were too great, further modifications of the *E. coli* genome would need to be performed. To make a tumor specific *E. coli* and use it to reduce tumor volume or growth in a patient would require a large amount of inventive effort, with each of the many intervening steps, upon effective reduction to practice, not providing any guarantee of success in the succeeding steps.

In view of the breadth of the claims and the lack of guidance provided by the specification as well as the unpredictability of the art, the skilled artisan would have required an undue amount of experimentation to make and/or use the claimed invention. Therefore, claims 1-13 are not considered to be enabled by the instant specification.

Claims 1-13 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The claims are drawn to a method for reducing volume or inhibiting growth of a solid tumor cancer, comprising administering to a patient having a solid tumor, a tumor specific *Escherichia coli* genetically engineered to express a suicide gene. The dependent claims limit the method to embodiments wherein the *E. coli* is attenuated, super-infective, or attenuated and super-infective. Further, the attenuated *E. coli* may expresses an altered lipid A molecule or induce TNF-α expression in monocytes or macrophages from about 1 to about 75 percent compared to non-attenuated microorganisms. The claims encompass embodiments wherein the *E. coli* is an auxotrophic mutant. The suicide gene of the *E. coli* may be selected from the group consisting of the open reading frame of pTK-Sec3, pCD-Sec1 and pSP-SAD4-5 or may encode p450 oxidoreductase, HSV thymidine kinase, *E. coli* cytosine deaminase, carboxypeptidase G2, β-glucuronidase, penicillin-V-amidase, penicillin-G-amidase, β-lactamase, β-glucosidase, nitroreductase and carboxypeptidase A. The suicide gene may be under the control of a constitutive promoter, an inducible promoter, or a tumor cell specific promoter.

The claimed method encompasses the provision of a set of *Escherichia coli* that are tumor specific. Further, the claimed method encompasses the provision of a set of *E. coli* that expresses an altered lipid A molecule, or induces TNF- α expression in monocytes or macrophages from about 1 to about 75 percent compared to non-attenuated microorganisms.

Moreover, the method encompasses the provision of *E. coli* that are attenuated, super-infective, or attenuated and super-infective. Wild type *E. coli* does not inherently possess any of these characteristics (e.g. tumor-specificity, see instant specification, page 96, 1st full paragraph; Table 18). Thus, the claims are drawn to a set of *E. coli* that has been modified to possess the claimed functional characteristics.

The specification defines tumor-specific strains as strains that are able to distinguish between a cancerous target cell and a non-cancerous counterpart cell so that the vector preferentially attaches to, infects and/or remains viable in the cancer cell (e.g. page 17, lines 22-29). Attenuation is defined as meaning both the modification of a microorganism to make it less pathogenic, and the modification of a microorganism so that a lower titer of that microorganism can be administered to a patient and still achieve comparable results as if one had administered a higher titer of the parental microorganism (e.g. page 16, lines 18-35). Super-infective strains are able to attach and/or infect a target cell more readily as compared to the wild type vector (e.g. page 17, lines 5-21).

To provide adequate written description and evidence of possession of a claimed genus, the specification must provide sufficient distinguishing identifying characteristics of the genus. The factors to be considered include disclosure of a complete or partial structure, physical and/or chemical properties, functional characteristics, structure/function correlation, and any combination thereof. The specification does not describe *E. coli* that is tumor specific. The specification describes methods of selecting for bacteria that are tumor specific (e.g. sections 6.1.1-6.1.4) but does not describe a single tumor specific strain of *E. coli* or the structure required to confer tumor specificity to a strain of *E. coli*. The specification describes one mutant,

firA$^-$, that has an altered lipid A molecule relative to wild type lipid A in that it contains a seventh fatty acid, a hexadecanoic acid, and has decreased lipid A 4' kinase activity (e.g. page 125, lines 20-32). Further, the specification refers to the teachings of Roy and Coleman, J. Bacteriol. Vol. 176, pages 1639-1646, 1994). Roy et al describe the lipid A biosynthetic pathway and note that the accumulation of a heptaacyl lipid A at the nonpermissive temperature is a common feature of lipid A biosynthetic mutants (e.g. page 1644, right column, 1st full paragraph). Further, the specification describes the effect of the *firA*$^-$ *Salmonella typhimurium* on the production of TNF-α in human macrophages *in vitro* (e.g. pages 126-129). No description is provided of any mutations that result in tumor specificity in combination with attenuation and/or super-infection. It is not possible for one to extrapolate from the examples provided for *Salmonella* those *E. coli* that would meet the functional limitation of the claims. One cannot envision the types or number of modifications that must be made to confer tumor specificity, attenuation, and/or super-infection to any *E. coli* strain.

The prior art does not appear to offset the deficiencies of the instant specification in that it does not describe a set of tumor specific *E. coli*. Karapetyan (EP 0564121, of record) teach three strains of *E. coli*, ATCC 55373, 55374 and 55375, which are oncolytic and hemolytic *in vitro* (e.g. page 3, line 5 to page 4, line 5; Table I). There is no description of the ability of the *E. coli* to distinguish between a cancerous target cell and a non-cancerous counterpart cell so that the vector preferentially attaches to, infects and/or remains viable in the cancer cell.

Given the very large genus of *E. coli* encompassed by the rejected claims, and given the limited description provided by the prior art and specification with regard to tumor specific *E. coli*, the skilled artisan would not have been able to envision a sufficient number of specific

embodiments that meet the functional limitations of the claims to describe the broadly claimed genus of tumor specific *E. coli* capable of being engineered to express a suicide gene and to effectively reduce the volume of a tumor or inhibit the growth of a tumor in a patient. Thus, there is no structural/functional basis provided by the prior art or instant specification for one of skill in the art to envision those *E. coli* strains that satisfy the functional limitations of the claims. Therefore, the skilled artisan would have reasonably concluded applicants were not in possession of the claimed invention for claims 1-13.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jennifer Dunston whose telephone number is 571-272-2916. The examiner can normally be reached on M-F, 9 am to 5 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Remy Yucel can be reached at 571-272-0781. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

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Jennifer Dunston
Examiner
Art Unit 1636

jad

TERRY MCKELVEY
PRIMARY EXAMINER